THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 64:175-177, 1975

Vol. 64, No. 3
Printed in U.S.A.

THE DETECTION OF CAROTENOID PIGMENTS IN HUMAN SKIN

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Carotenoid pigments were extracted chemically from both epidermis and dermis obtained from non-carotenemic individuals at autopsy. Absorption maxima characteristic of beta-carotene were found in the extracts of specimens of epidermis following cantharidin application in volunteers made carotenemic by the ingestion of beta-carotene (180 mg/day for 10 weeks). These maxima were absent in the extracts of epidermis obtained from the volunteers before beta-carotene ingestion.

Carotenoderma was first described in 1919 by Hess and Meyers [1]. Many studies of the distribution of carotenoid pigments in man have determined the amounts of these pigments in many organs, including subcutaneous fat, but we could find no report of the chemical extraction of carotenoids from human skin. The presence of carotenoids in skin has been deduced mainly by visual observation of yellow-orange color of skin, or by reflection spectrophotometry [2,3]. Dohi and Ohno [4] examined microscopically a section of skin from a carotenemic patient, and observed a yellow color in the horny layer. Grof et al [5] scraped some horny layer from the palms, soles, and knees of a carotenemic patient and were able to extract carotene from the scrapings. The only reports of chemical extraction of carotenoids from skin were those dealing with the extraction from chicken skin, such as the studies of Smith and Perdue [6] and Livingston et al [7]. Whether carotenoids are chemically identifiable in human skin has become of particular concern after recent demonstrations that carotenoids are useful in ameliorating the photosensitivity of erythropoietic protoporphyria [8]. Therefore, human skin was studied to determine chemically whether carotenoids were present.

MATERIALS AND METHODS

Specimens of skin were obtained at autopsy from non-carotenemic fair-skinned Caucasians. These specimens were diced, extracted, and saponified in a solution of 12% KOH in abolute methanol for 30 to 60 min at 40°C. After cooling, the methanolic solutions were extracted with petroleum ether (boiling point 35–60°C). The petroleum ether layers were pooled, washed free of alkali with water, evaporated to dryness, and the residue taken up in petroleum ether. Some of the extracts were chromatographed on Eastman Kodak silica gel thin-layer sheets, using 10% absolute ethanol in petroleum ether as the developing solvent. The absorption spectra were determined in a Cary model 14 recording spectrophotometer.

RESULTS

Figure 1 shows the absorption spectrum of the petroleum ether extract of whole skin. It can be seen that faint, but characteristic, absorption maxima for carotenoids are present.

To determine the carotenoid content of epidermis and dermis, we separated whole skin into these layers by the scraping method [9] or the heating method [10], and then diced and extracted the layers as above. Figures 2 and 3 show that both epidermis and dermis contained carotenoids. A spectrum of pure beta-carotene is included in Figure 2 for comparison. The spectra show a mixture of beta-carotene and other carotenoids. The subjects from whom the skin was obtained for the extractions shown in Figures 1 to 3 were probably eating various kinds of vegetables, containing different carotenoids. The concentration of carotene in the epidermis (Fig. 2) was found to be 0.21 μg/gm wet wt epidermis, and that of the dermis (Fig. 3) was found to be 0.07 μg carotenoids/gm wet wt dermis. Thus the epidermis seems to contain more carotenoids on a weight basis than does the dermis.

Attempts were made to develop an assay for carotene in the skin. Cantharidin blisters were induced in 4 male volunteers before and after 8 weeks of ingestion of 180 mg/day of beta-carotene to see whether we could detect the presence of carotenoids in the sheet of epidermis produced by the blister. Cantharidin solution (Cantharone, Ingram Pharmaceutical Co., San Francisco, Calif.) was painted on the skin of the lateral abdominal wall in a circle approximately 25 mm in diameter. Blisters formed 24 to 30 hr afterward. Circular areas of epidermis ranging from 22 to 24 mm in

Manuscript received July 12, 1974; in revised form October 4, 1974; accepted for publication October 7, 1974.

This work was supported by research grants AM 14545 from the National Institute of Arthritis, Metabolism, and Digestive Diseases (M.M.M.) and CA 05003 15 from the National Cancer Institute (M.A.P.).

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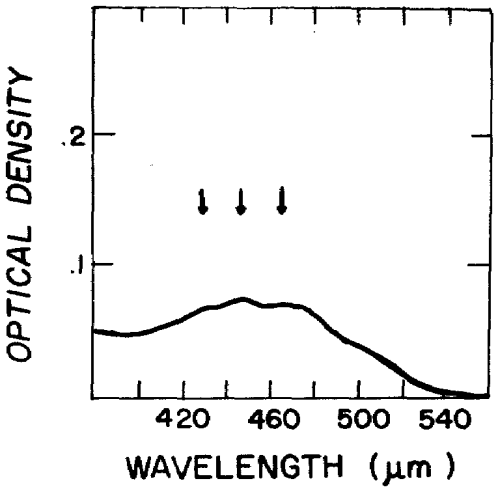

FIG. 1. Absorption spectrum of petroleum ether extract of whole skin (2.46 gm) of a non-carotenemic individual, obtained at autopsy. *Arrows* indicate absorption maxima characteristic of carotenoid pigments.

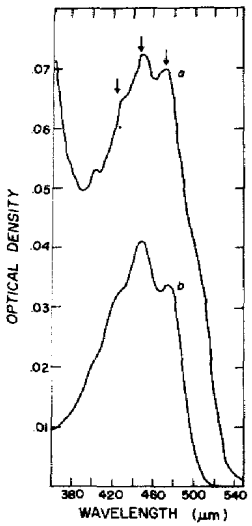

FIG. 2. Absorption spectrum of petroleum ether extract of epidermis (0.91 gm) of a non-carotenemic individual obtained at autopsy (*a*), and the absorption spectrum of pure beta-carotene in petroleum ether (*b*). *Arrows* indicate absorption maxima characteristic of carotenoid pigments.

diameter were removed, diced, and extracted as above. Figure 4 shows faint but definite maxima, indicating the presence of carotenoids in the extract of the epidermis removed after 10 weeks of beta-carotene ingestion from one of the volunteers. Figure 4 also shows the absence of these maxima in the extract of this same volunteer's blister epidermis which had been obtained before the start of beta-carotene ingestion. The concentration of carotenoids was found to be 0.9 μg/gm of blister epidermis. The volunteer's blood carotenoid level at the time the blister was induced was 650 μg%.

DISCUSSION

These findings confirm chemically the visual and reflection spectrophotometric observations of the presence of carotenoid pigments in human skin, and extend the observations further to show that carotenoids are present in both epidermis and dermis, and in greater concentration in the former. The results also show that present methods make it difficult to do carotenoid determinations on skin repeatedly. In the non-carotenemic subjects, pieces of skin measuring several inches in length

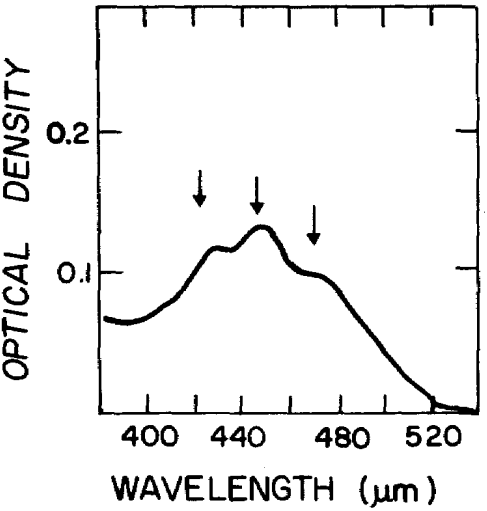

FIG. 3. Absorption spectrum of petroleum ether extract of dermis (4.03 gm) of a non-carotenemic individual obtained at autopsy. *Arrows* indicate absorption maxima characteristic of carotenoids.

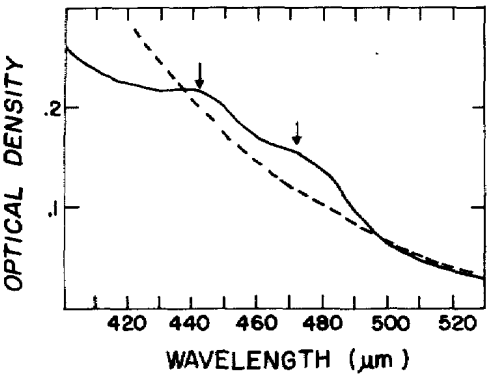

FIG. 4. Absorption spectrum of petroleum ether extract of epidermis obtained from a volunteer by cantharidin blister formation before (-----) and after (——) 10 weeks of ingestion of 180 mg/day of beta-carotene (Hoffmann-La Roche "beadlet" preparation). The pieces of epidermis weighed 26.1 and 17.1 mg, respectively.

were needed to produce the spectra shown in Figures 1 to 3; specimens this size are not likely to be obtained readily from patients receiving carotenoid treatment [8,11]. Even in the carotenemic individuals, in whom cantharidin blisters were induced while the men were carotenemic, 2 of 4 samples did not show the characteristic absorption bands indicative of the presence of carotenoids, even though all volunteers had blood carotenoid values over 600 μg%. In addition, the induction of a catharidin blister is a fairly painful process, and would not be tolerated by patients as a repetitive procedure. Thus, the use of skin carotene determinations for the assessment of carotenodermia must await the development of a highly sensitive technique in which small amounts of epidermis and dermis can be removed for the quantitative determination of carotene in a manner acceptable to the patient.

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